

REMARKS

It is respectfully submitted that the amendments submitted herewith function only to insert the sequence listing and appropriate sequence identifiers into the text of the present application to comply with 37 C.F.R. §1.821 to 1.825. These amendments are made without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents.

It is respectfully asserted that the sequence disclosure contained in the application now fully complies with the requirements set forth in 37 C.F.R. § 1.821 to § 1.825.

It is respectfully submitted that the Sequence Listing conforms to the requirements of 37 C.F.R. §1.823(b). The Statements required by 37 C.F.R. §1.821(f) and (g) are set forth below.

Pursuant to 37 C.F.R. §1.821 (g), the undersigned hereby states that this submission, filed in accordance with 37 C.F.R. §1.821 (g), does not contain new matter.

Pursuant to 37 C.F.R. §1.821 (f), the undersigned hereby states that the content of the paper and computer readable copies of the Sequence Listing submitted in accordance with 37 C.F.R. §1.821 (c) and (e), respectively, are the same.

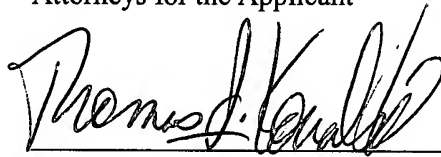
CONCLUSION

In view of the amendments, remarks and enclosures herein, it is respectfully submitted that the application now complies with all requirements set forth in the Notice. Accordingly, reconsideration and withdrawal of the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures ("Notice to Comply") is respectfully requested.

Respectfully submitted,

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Enclosures: Paper and Diskette copies of Sequence Listing
Copy of Notice to Response To Notice To Comply
Return receipt postcard

"VERSION WITH MARKINGS TO SHOW CHANGES MADE"

Pages 37, line 23:

Plasmid constructions. Standard molecular biology procedures were used (Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) Molecular Cloning. A Laboratory Manual (Cold Spring Harbor Press, N.Y.)). The schematic organization of the plasmids used in this study is represented Figure 2. *Gp31* gene was PCR (polymerase chain reaction) amplified using two oligonucleotides 5'-C TTC AGA CAT ATG TCT GAA GTA CAA CAG CTA CC-3' (SEQ ID NO: 1) and 5' -TAA CGG CCG TTA CTT ATA AAG ACA CGG AAT AGC-3' (SEQ ID NO: 2) producing a 358bp DNA using pSV25 (van der Vies, S., Gatenby, A. & Georgopoulos, C. (1994) Nature 368, 654-656) as template. The DNA sequence of a part of the mobile loop of Gp31 (residues 25 to 43) was removed by PCR, as described (Hemsley, A., Arnheim, N., Toney, M.D., Cortopassi, G. & Galas, D. J. (1989) Nucleic Acids Res. 17, 6545-6551), using oligonucleotides 5' -**GGA** GAA GTT CCT GAA CTG-3' (SEQ ID NO: 3) and 5'-**GGA TCC** GGC TTG TGC AGG TTC-3' (SEQ ID NO: 4), creating a unique *Bam*H I site (bold characters). *GroEL* gene minichaperone (corresponding to the apical domain of GroEL, residues 191 to 376; (Zahn, R., Buckle, A.M., Peret, S., Johnson, C. M. J., Corrales, F. J., Golbik, r. & Fersht, A. R. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 15024-15029)) was amplified by PCR using oligonucleotides, containing a *BAM*H I site (underlined), 5'-TTC GGA TCC GAA GGT ATG CAG TTC GAC C- 3' (SEQ ID NO: 5) and 5'-GTT GGA TCC AAC GCC GCC TGC CAG TTT C- 3' (SEQ ID NO: 6) and cloned into the unique *Bam*H I site of pRSETA-Gp31Δloop sequence. The single ring GroEL_{SR1} mutant contains four amino acid substitutions (R452E, E461A, S463A, and V464A) into the equatorial interface of Gro EL, which prevent the formation of double rings (Weissman, J. S., Hohl, C. M., Kovalenko, O., Kashi, Y., Chen, S., Braig, K., Saibil, H.R., Fenton, W.A. & Horwich, A.L. (1995) Cel 83, 577-587). The corresponding mutations were introduced into *groEL* by PCR (Hemsley, A., Arnheim, N., Toney, M.D., Cortopassi, G. & Galas, D. J. (1989) Nucleic Acid Res. 17, 6545-6551) using oligonucleotides 5'-TGA GTA CGA TCT GTT CCA GCG GAG CTT CC' (SEQ ID NO: 7) and 5' -**ATT GCG GCG** AAG CGC CGG CTG C TG TTG CTA ACA CCG-3' (SEQ ID NO: 8) and pRSETA-*Eag* I GroEL or GroESL vectors (Chatellier, J., Hill, F., Lund, P. & Fersht, A.R. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 9861-9866) as template; silent mutations, in respect to the codon usage in *E. coli*, create a unique *Mfe* I (bold characters) and *Nae* I (underlined).

GroEL (E191G; *groEL44* allele) gene was PCR amplified from *E. coli* SV2 strain (Zeilstra-Ryalls, J., Fayet, O., Baird, L. & Georgeopoulos, C. (1993) *J. Bacteriol.* 175, 1134-1143) using two oligonucleotides 5'-T AGC TGC CAT ATG GCA GCT AAA GAC GTA AAA TTC GG-3' (SEQ ID NO: 9) and 5'-ATGTAA CGG CCG TTA CAT CAT GCC GCC CATGCC ACC-3' (SEQ ID NO: 10) producing a 1,659 bp DNA with unique sites for Nde I and Eag I (underlined). The different genes were subcloned into the unique Nde I and Eag I unique sites of pACYC184, pJC and pBAD30 (Guzman, L.-M., Belin, D., Carson, M.J. & Beckwith, J. (1995) *J. Bacteriol.* 177, 4121-4130) vectors (Chatellier, J., Hill, F., Lund, P. & Fersht, A.R. (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95, 9861-9866). A colony-based PCR procedure was used to identify the positive clones (Chatellier, J., Mazza, A., Brousseau, R. & Vernet, T. (1995) *Analyt. Biochem.* 229, 282-290). PCR cycle sequencing using fluorescent dideoxy chain terminators (Applied Biosystems) were performed and analysed on an Applied Biosystems 373A Automated DNA. All PCR amplified DNA fragments were sequenced after cloning.

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Plasmid constructions Standard molecular biology procedures were used (Sambrook et al., 1989). The plasmid pRSETA encoding GroES gene as been described (Chatellier et al. 1998. 1998 In vivo activities of GroEL minichaperones. *Proc. Natl. Acad. Sci. USA* 95, 9861-9866). The GroES mutant Gly24Trp was generated by polymerase chain reaction (PCR), as described (Helmsley et al., 1989 A simple method for site directed mutagenesis using the polymerase chain reaction. *Nucl. Acids Res.* 17, 6545-6551) using the template pRSETA encoding GroES (Chatellier et al., 1998) and the oligonucleotides 5' -C GGC TGG ATC GTT CTG ACC G-3' (SEQ ID NO: 11) and 5' -GC AGA TTT AGT TTC AAC TTC TTT ACG-3' (SEQ ID NO: 12), creating a Nae I site (Bold characters).

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The DNA sequence encoding a part of the mobile loop of GroES (residues 16 to 33) was removed by PCR, as described (Hemsley et al., 1989), using the oligonucleotides 5' - TCC GGC TCT GCA GCG G- 3' (SEQ ID NO: 13) and 5' - TCC AGA GCC AGT TTC AAC TTC TTT ACG C -3' (SEQ ID NO: 14), creating a unique BamH I site (bold characters) and the vector pRSET A-Gro ESΔloop.